

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 6 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Amendment to the Claims:

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claims 1 to 30 (canceled)

Claim 31 (currently amended): A method of generating a nucleic acid that encodes [[encoding]] a polypeptide having a polymerase activity comprising:

obtaining a nucleic acid comprising a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity, or its complement;

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;

expressing the variant nucleic acid to generate a polypeptide; and
screening the polypeptide for a polymerase activity, thereby generating a nucleic acid encoding a polypeptide having a polymerase activity.

Claim 32 (currently amended): The method of claim 31, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, [[gene site saturated mutagenesis (GSSM)]] Gene Site Saturation MutagenesisTM (GSSMTM), synthetic gene reassembly and any combination thereof.

Claim 33 (original): The method of claim 31, wherein the modifications are introduced by error-prone PCR.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 7 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 34 (original): The method of claim 31, wherein the modifications are introduced by shuffling.

Claim 35 (original): The method of claim 31, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 36 (original): The method of claim 31, wherein the modifications are introduced by assembly PCR.

Claim 37 (original): The method of claim 31, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 38 (original): The method of claim 31, wherein the modifications are introduced by in vivo mutagenesis.

Claim 39 (original): The method of claim 31, wherein the modifications are introduced by cassette mutagenesis.

Claim 40 (original): The method of claim 31, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 41 (original): The method of claim 31, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 42 (original): The method of claim 31, wherein the modifications are introduced by site-specific mutagenesis.

Claims 43 to 52 (canceled)

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 8 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 53 (currently amended): A method of generating a nucleic acid that encodes [[encoding]] a polypeptide having a polymerase activity comprising:
obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or its complement;
modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;
expressing the variant nucleic acid to generate a polypeptide; and
screening the polypeptide for a polymerase activity, thereby generating a nucleic acid encoding a polypeptide having a polymerase activity.

Claim 54 (currently amended): The method of claim 53, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, [[gene site saturated mutagenesis (GSSM)]] Gene Site Saturation Mutagenesis™ (GSSM™) and any combination thereof.

Claim 55 (previously presented): The method of claim 53, wherein the modifications are introduced by error-prone PCR.

Claim 56 (previously presented): The method of claim 53, wherein the modifications are introduced by shuffling.

Claim 57 (previously presented): The method of claim 53, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 58 (previously presented): The method of claim 53, wherein the modifications are introduced by assembly PCR.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 9 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 59 (previously presented): The method of claim 53, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 60 (previously presented): The method of claim 53, wherein the modifications are introduced by in vivo mutagenesis.

Claim 61 (previously presented): The method of claim 53, wherein the modifications are introduced by cassette mutagenesis.

Claim 62 (previously presented): The method of claim 53, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 63 (previously presented): The method of claim 53, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 64 (previously presented): The method of claim 53, wherein the modifications are introduced by site-specific mutagenesis.

Claim 65 (currently amended): A method of generating a nucleic acid that encodes [[encoding]] a polypeptide having a polymerase activity comprising:
obtaining a nucleic acid comprising a fragment of at least 30 consecutive nucleotides of a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity, or its complement;
modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;
expressing the variant nucleic acid to generate a polypeptide; and

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 10 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

screening the polypeptide for a polymerase activity, thereby generating a nucleic acid encoding a polypeptide having a polymerase activity.

Claim 66 (currently amended): The method of claim 65, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, [[gene site saturated mutagenesis (GSSM)]] Gene Site Saturation Mutagenesis™ (GSSM™) and any combination thereof.

Claim 67 (previously presented): The method of claim 65, wherein the modifications are introduced by error-prone PCR.

Claim 68 (previously presented): The method of claim 65, wherein the modifications are introduced by shuffling.

Claim 69 (previously presented): The method of claim 65, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 70 (previously presented): The method of claim 65, wherein the modifications are introduced by assembly PCR.

Claim 71 (previously presented): The method of claim 65, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 72 (previously presented): The method of claim 65, wherein the modifications are introduced by in vivo mutagenesis.

Claim 73 (previously presented): The method of claim 65, wherein the modifications are introduced by cassette mutagenesis.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 11 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 74 (previously presented): The method of claim 65, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 75 (previously presented): The method of claim 65, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 76 (previously presented): The method of claim 65, wherein the modifications are introduced by site-specific mutagenesis.

Claim 77 (currently amended): A method of generating a nucleic acid that encodes [[encoding]] a polypeptide having a polymerase activity comprising:

obtaining a nucleic acid comprising a fragment of at least 30 consecutive nucleotides of a sequence as set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity or its complement;

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;

expressing the variant nucleic acid to generate a polypeptide; and

screening the polypeptide for a polymerase activity, thereby generating a nucleic acid encoding a polypeptide having a polymerase activity.

Claim 78 (currently amended) The method of claim 77, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, [[gene site saturated mutagenesis (GSSM)]] Gene Site Saturation Mutagenesis™ (GSSM™) and any combination thereof.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 12 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 79 (previously presented) The method of claim 77, wherein the modifications are introduced by error-prone PCR.

Claim 80 (previously presented) The method of claim 77, wherein the modifications are introduced by shuffling.

Claim 81 (previously presented) The method of claim 77, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 82 (previously presented) The method of claim 77, wherein the modifications are introduced by assembly PCR.

Claim 83 (previously presented) The method of claim 77, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 84 (previously presented) The method of claim 77, wherein the modifications are introduced by in vivo mutagenesis.

Claim 85 (previously presented) The method of claim 77, wherein the modifications are introduced by cassette mutagenesis.

Claim 86 (previously presented) The method of claim 77, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 87 (previously presented) The method of claim 77, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 88 (previously presented) The method of claim 77, wherein the modifications are introduced by site-specific mutagenesis.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 13 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 89 (previously presented): The method of claim 31 or claim 65, wherein the polymerase activity is a thermostable polymerase activity.

Claim 90 (previously presented): The method of claim 89, wherein the thermostable polymerase activity comprises activity at a temperature in a range from about 95°C to 113°C.

Claim 91 (currently amended): The method of claim 31 or claim 65, wherein the [[polymerase activity comprises a]] polypeptide also has 3'→ 5' exonuclease activity.

Claim 92 (previously presented): The method of claim 31 or claim 65, wherein the polymerase activity comprises an activity that can function under conditions of high salinity.

Claim 93 (previously presented): The method of claim 31 or claim 65, wherein the nucleic acid encoding the polymerase has a high guanine-cytosine (GC) content.

Claim 94 (previously presented): The method of claim 31 or claim 65, wherein the polymerase activity comprises amplifying a template sequence during PCR amplification procedures.

Claim 95 (previously presented): The method of claim 31 or claim 65, comprising obtaining a nucleic acid comprising a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.

Claim 96 (previously presented): The method of claim 95, comprising obtaining a nucleic acid comprising a sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 14 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

Claim 97 (previously presented): The method of claim 96, comprising obtaining a nucleic acid comprising a sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.

Claim 98 (new): A method of generating a nucleic acid that encodes a polypeptide having polymerase activity comprising:

obtaining a nucleic acid comprising a sequence set forth in SEQ ID NO:1, or its complement;

modifying one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence, or adding one or more nucleotides to the sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;

expressing variant nucleic acid to generate a polypeptide; and

screening the polypeptide for polymerase activity, thereby generating a nucleic acid encoding a polypeptide having polymerase activity.

Claim 99 (new): The method of claim 98, wherein the modifying one or more nucleotides in the sequence results in the generation of a polypeptide also having 3'→ 5' exonuclease activity.

Claim 100 (new): The method of claim 98, wherein the modifying one or more nucleotides in the sequence results in the generation of a polypeptide having thermostable polymerase.

Claim 101 (new): A method of generating a nucleic acid that encodes a polypeptide having polymerase activity comprising:

obtaining a nucleic acid comprising a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity, or its complement;

Applicant : Walter Callen et al.
Serial No. : 09/656,309
Filed : September 6, 2000
Page : 15 of 22

Attorney's Docket No.: 56446-20016.20/-
027003 /D1350-2

modifying one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence, or adding one or more nucleotides to the sequence, to generate a variant nucleic acid that encodes a polypeptide having polymerase activity;

expressing variant nucleic acid to generate a polypeptide; and

screening the polypeptide for a polymerase activity, thereby generating a nucleic acid encoding a polypeptide having a polymerase activity,

wherein modifying one or more nucleotides in the sequence comprises use of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, synthetic gene reassembly or [[gene site saturated mutagenesis (GSSM)]] Gene Site Saturation Mutagenesis™ (GSSM™).

Claim 102 (new) The method of claim 31, wherein the modifications are introduced by ligation reassembly.

Claim 103 (new) The method of claim 31, wherein the modifications are introduced by Gene Site Saturation Mutagenesis™ (GSSM™).

Claim 104 (new) The method of claim 53, wherein the modifications are introduced by ligation reassembly.

Claim 105 (new) The method of claim 53, wherein the modifications are introduced by Gene Site Saturation Mutagenesis™ (GSSM™).

Claim 106 (new) The method of claim 65, wherein the modifications are introduced by ligation reassembly.

Claim 107 (new) The method of claim 65, wherein the modifications are introduced by Gene Site Saturation Mutagenesis™ (GSSM™).